

HEMOSTATIC ADAPTATIONS FOLLOWING A HIGH-INTENSITY INTERVAL TRAINING
INTERVENTION IN HEALTHY MEN

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ABSTRACT

THESIS: Hemostatic Adaptations Following a High-Intensity Interval Training Intervention in Healthy Men

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High intensity interval training (HIIT) is a novel mode of exercise training that has been shown to improve several components of health in various healthy and diseased populations. **Purpose:** The purpose of the present study was to examine hemostatic adaptations in healthy adult men following four and eight weeks of a HIIT intervention. **Methods:** Twenty-one healthy but sedentary men (age: 25 ± 5 yrs; BMI: 26.7 ± 6.2 kg/m²) volunteered to participate in the present study. Subjects completed eight weeks of HIIT that included three to six 'all out' Wingate tests three days/week. Overall blood coagulation was assessed at baseline, following four weeks, and following eight weeks by clotting times of activated partial thromboplastin time (APPT) and prothrombin time (PT), and plasma concentration of fibrinogen. Plasma was obtained from whole blood samples taken at rest. A repeated measures ANOVA was used to compare the overall coagulation potential. Significance was set to $p < 0.05$. **Results:** There were no significant differences between resting heart rate, resting blood pressure, APTT (baseline: 43.0 ± 5.4 ; 4w: $42.7 \pm$

5.1; 8w: 44.2 ± 6.2), and PT (baseline: 13.0 ± 0.9 ; 4w: 12.9 ± 0.6 ; 8w: 13.1 ± 0.8), after 4w or 8w of HIIT. Fibrinogen concentrations significantly decreased from baseline to 4w ($p < 0.05$) and significantly increased from 4w to 8w ($p < 0.05$). **Conclusion:** Although beneficial fibrinogen changes were seen following four weeks of training, these findings were reversed after eight weeks. These observations suggest that HIIT may elicit improvements in coagulation potential after four weeks, but continued training may lead to elevated coagulation and/or inflammation via fibrinogen, which is recognized as a key regulator of inflammation and disease.

CHAPTER I

INTRODUCTION

Although the existing body of knowledge and research on cardiovascular disease (CVD) has grown tremendously over the past several years, CVD remains the most common cause of death in the world today [1]. A study by Murphy et al. showed that CVD or a CVD related event accounted for 24.2% of the total deaths in 2010 [1]. Several peer-reviewed articles touch on how CVD and its predictors can be prevented, with the most common task being regular physical activity [[2, 3]. It is well known that exercising regularly will help prevent CVD and several CVD complications.

Exercise and Thrombosis

The majority of cardiovascular events, including approximately 80% of acute myocardial infarctions, are initiated by a blood clot, or thrombus, that occludes arterial blood flow [4]. The ability of one's blood to clot, or the coagulation potential, has been directly related to the onset of CVD as a thrombus may occur [5]. The prediction of

coronary risk can be determined by several factors (e.g., Factor VIII [FVIII] and fibrinogen) as these factors may promote a procoagulant state of the hemostatic system [2]. It is theorized that exercise training reduces one's overall coagulation potential, as this is one of the reasons that regular exercise is cardioprotective.

Standard exercise guidelines recommend 150 minutes per week of moderate-intensity aerobic physical activity, typically occurring in 20-60 minute bouts of continuous training, taking place 3-5 days per week [6, 7]. Previously published studies utilize this recommendation from the American College of Sports Medicine but have produced inconsistent results with regard to coagulation potential [8-11]. Several modes of exercise training show an increased coagulation potential [12-14], while others show a decrease [10, 11, 15] or no change in coagulation activity following training [16]. These equivocal results related to exercise training support the need for further investigations of training regimens that may differ from traditional recommendations.

High-Intensity Interval Training

High-intensity interval training (HIIT) involves short, intermittent bouts of vigorous physical activity, interspersed with brief periods of passive or low-intensity active recovery. Common HIIT protocols utilize 5-30 seconds of high-intensity exercise, followed by a brief recovery period, repeated 3-6 times. Various HIIT protocols have been shown to improve markers of aerobic physical fitness in various healthy and diseased populations [17-21]. Also, HIIT has been shown to improve endothelial function, resting blood pressure, and left ventricular morphology [17, 20, 22], each of which is related to coagulation potential [23-25].

Previous research indicates that the magnitude of coagulation responses to acute exercise are positively associated with intensity [26], leading to the speculation that the moderate intensities used in traditional exercise training may not stimulate the hemostatic system in such a way that would lead to beneficial changes in coagulation potential. Blood clotting adaptations following a HIIT regimen are unknown.

Statement of the Problem

Clotting potential of one's blood has been related to several adverse cardiovascular events [5]. Furthermore, exercise training is expected to reduce one's thrombotic risk by lowering overall coagulation potential [26, 27]. Despite the large body of evidence supporting the benefits of regular exercise and the known dose-response relationship between activity and disease, there is no consensus regarding the required dose of exercise needed to promote desired physiological adaptations [28, 29].

Specific Aims

The specific aims of this study were:

- 1) To determine whether healthy adults experience increased or decreased levels of FVIII and fibrinogen following four and eight weeks in an eight week HIIT intervention.
- 2) To determine whether healthy adults experience increased or decreased prothrombin time (PT) and activated partial thromboplastin time (APTT) following four and eight weeks in an eight week HIIT intervention.

Hypotheses

It was hypothesized that sedentary, healthy men will experience a progressive decrease in FVIII and fibrinogen levels as training volume increases throughout the eight week HIIT intervention. It was also hypothesized that sedentary, healthy men will experience a progressive increase in clotting times throughout the eight week HIIT intervention.

Importance of the Study

There is controversy regarding the effect of traditional endurance training on hemostatic variables, especially those of coagulation potential. Understanding the proper dose-response relationship of regular exercise training to promote desired physiological outcomes is important in both research and applied settings. However, the effects of a HIIT intervention on hemostatic variables have yet to be examined. The results from this research study will demonstrate novel and valuable findings to add to the scientific literature and yield a deeper understanding of exercise training adaptations on the hemostatic system.

Delimitations

Twenty-one non-smoking men between 18-45 years old volunteered for this study. Subjects were sedentary (less than two days, 30 minutes a day, of aerobic physical activity per week in the past three months) and free from any known cardiovascular or metabolic disease. Subjects also weighed more than 50 kg (110 lbs) and had no physical limitations that would prevent them from doing strenuous anaerobic exercise.

Subjects considered "high risk" according to American College of Sports Medicine guidelines were ineligible to participate. High risk was defined as any known cardiovascular, pulmonary, and/or metabolic disease as well as any of the following major signs or symptoms of the aforementioned diseases: angina, abnormal dyspnea, syncope, orthopnea/paroxysmal nocturnal dyspnea, ankle edema, tachycardia, intermittent claudication, and unusual fatigue with usual activities. Use of prescription medications that may influence performance and/or hemostatic parameters under investigation were cause for exclusion from the study. Any enrolled subjects who exhibited symptoms indicative of cardiovascular distress during participation were eliminated from further involvement in the study.

Definition of Terms

- 1) Hemostasis – Pertaining to the movement or lack of movement of the blood.
- 2) Coagulation – The process of the formation of a blood clot.
- 3) Thrombosis – The formation or presence of a blood clot in the circulatory system.
- 4) Sedentary – Less than two days, 30 minutes per day, of aerobic physical activity within the past three months.
- 5) High-intensity interval training (HIIT) – Characterized by short, intermittent bouts of vigorous physical activity, interspersed with brief periods of passive or low intensity active recovery.

CHAPTER II

REVIEW OF LITERATURE

Cardiovascular disease (CVD) is the most common cause of mortality in the United States, and was responsible for one out of every three deaths in 2010 [30]. The most common types of CVD, including myocardial infarction, stroke, and sudden cardiac death are caused by the formation of an occlusive thrombus [4]. Such thrombotic events are related to blood coagulation and fibrinolytic activity [5]. The hemostatic system is affected by several different mechanisms including age, sex, smoking, obesity, and physical activity [26, 27, 31-33].

Previous research indicates that regular exercise training has been shown to be a plausible contributor to the reduction of cardiac events because of its direct effects on the body's vasculature and the shear stress that is placed on the endothelium [23]. However, research regarding the hemostatic system and endurance training has produced equivocal results. Some studies show an increase in coagulation potential [12-14], others show a decrease in coagulation potential [10, 11, 15], and some show no change in coagulation

potential [16]. While several studies have shown endurance training to be most beneficial to the hemostatic system, no research has shown the effect of high-intensity interval training (HIIT) on the hemostatic system. Despite the recognized benefits of regular exercise and the known dose-response relationship between activity and disease, there is no consensus regarding the dose of exercise needed to promote desired physiological adaptations [28, 29]. Thus, the purpose of this investigation was to determine whether healthy adults experience an increased or decreased coagulation potential following four and eight weeks of a HIIT intervention. Based upon previous findings and the theory that exercise training reduces blood coagulation, it is hypothesized that HIIT will reduce coagulation potential following four and eight weeks of HIIT.

Blood Coagulation

Although complex in nature, the coagulation cascade is an important mechanism for preventing excessive blood loss from an injured blood vessel [34]. Following injury to a blood vessel, vasoconstriction is the first responsive mechanism to control for blood loss. However, because vasoconstriction alone is not enough to completely stop blood flow, the formation of a clot is necessary. Clot formation begins when platelets flowing through the blood vessel attach to exposed collagen and become activated. Upon activation, the platelets release adenosine diphosphate and thromboxane A₂. Adenosine diphosphate attracts more platelets into the injured area and thromboxane A₂ promotes vasoconstriction and platelet aggregation. This mechanism works in positive feedback fashion to form the platelet plug. Following the formation of the platelet plug, the coagulation cascade takes over to form the rest of the blood clot. A major protein of blood

coagulation, fibrin, is recruited to form the cross-linked fibrin clot. The cross-linked fibrin clot is the final piece of the coagulation cascade.

The coagulation cascade consists of three pathways: the intrinsic pathway, the extrinsic pathway, and the common pathway. A damaged surface and exposed proteins found in the blood stream including high-molecular-weight kininogen, prekallikrein, and factor XII initiate the intrinsic pathway. On the contrary, the extrinsic pathway is initiated by trauma and the exposure of tissue factor from the subendothelium. Tissue factor is a cell membrane protein that does not travel in the blood stream. The intrinsic and extrinsic pathways lead to the formation of factor X, which is where the common pathway begins. The common pathway is initiated by factor X and completed when a cross-linked fibrin clot is formed. The three pathways mentioned above work in conjunction to form a blood clot. Several enzymes work together and in positive feedback fashion to activate other substrates and stimulate the formation of other enzymes.

The intrinsic pathway is very important for the amplification of the coagulation cascade. While the initial activation of factor X is from the extrinsic pathway, the intrinsic pathway amplifies this activation [35, 36]. The formation of the primary complex on collagen begins with three important proteins: high-molecular-weight kininogen, prekallikrein, and factor XII. Prekallikrein is converted to kallikrein and factor XII is activated when it comes in contact with negatively charge surfaces. Factor XIIa causes factor XI to become activated. Next, factor XIa converts factor IX into its activated form, factor IXa. Factor IXa then needs the help of factor VIIIa, converted from factor VIII, to activate factor X to factor Xa. Factor Xa is the final product of the intrinsic pathway.

The extrinsic pathway is less complex. Following damage to a blood vessel, factor VII leaves the circulation and comes in contact with a negatively charged surface, such as tissue factor or collagen from within the arterial wall. Tissue factor is released from damaged tissue and at the same time, factor VII is converted to its active form, factor VIIa. Factor VIIa combines with tissue factor to convert factor X to factor Xa. This is where the intrinsic and extrinsic pathways meet to form the common pathway.

The final pathway of the coagulation cascade, the common pathway, contains three major reactions that each represent a rate limiting step of the coagulation cascade. Initially, factor X is activated via the products of the intrinsic and extrinsic pathways through their respective enzymes. Next, factor Xa converts prothrombin to thrombin with the help of factor Va, which was converted from factor V. Finally, thrombin then converts fibrinogen to fibrin. Thrombin plays an important role in positive feedback for the entire coagulation process as it accelerates the production of factor XIa, factor VIIIa, and factor Va. This amplifies the cascade to produce enough fibrin, the final protein in the formation of a blood clot, in a short amount of time [34].

Coagulation potential is generally measured in four common ways: measures of activated partial thromboplastin time (APTT) and prothrombin time (PT), and measures of protein activity of fibrinogen and factor VIII [26, 27]. APPT measures the time it takes to get through the intrinsic pathway and PT measures the time it takes to get through the extrinsic pathway. Furthermore, measures of fibrinogen and factor VIII are based on concentration levels in the blood. The more concentrated these proteins, the greater the coagulation potential.

The coagulation cascade, formed through the intrinsic, extrinsic, and common pathways, can be seen below in Figure A. Note that the three pathways of the coagulation cascade are not equally exclusive of one another. Enzymes from one pathway may activate substrates in another pathway and vice versa. Many of these enzymes work in positive feedback fashion, which helps to amplify the cascade and ensure that adequate amounts of fibrin are formed throughout. Overall, a hemostatic plug that occludes the flow of blood in an injured vessel is formed through this cascade.

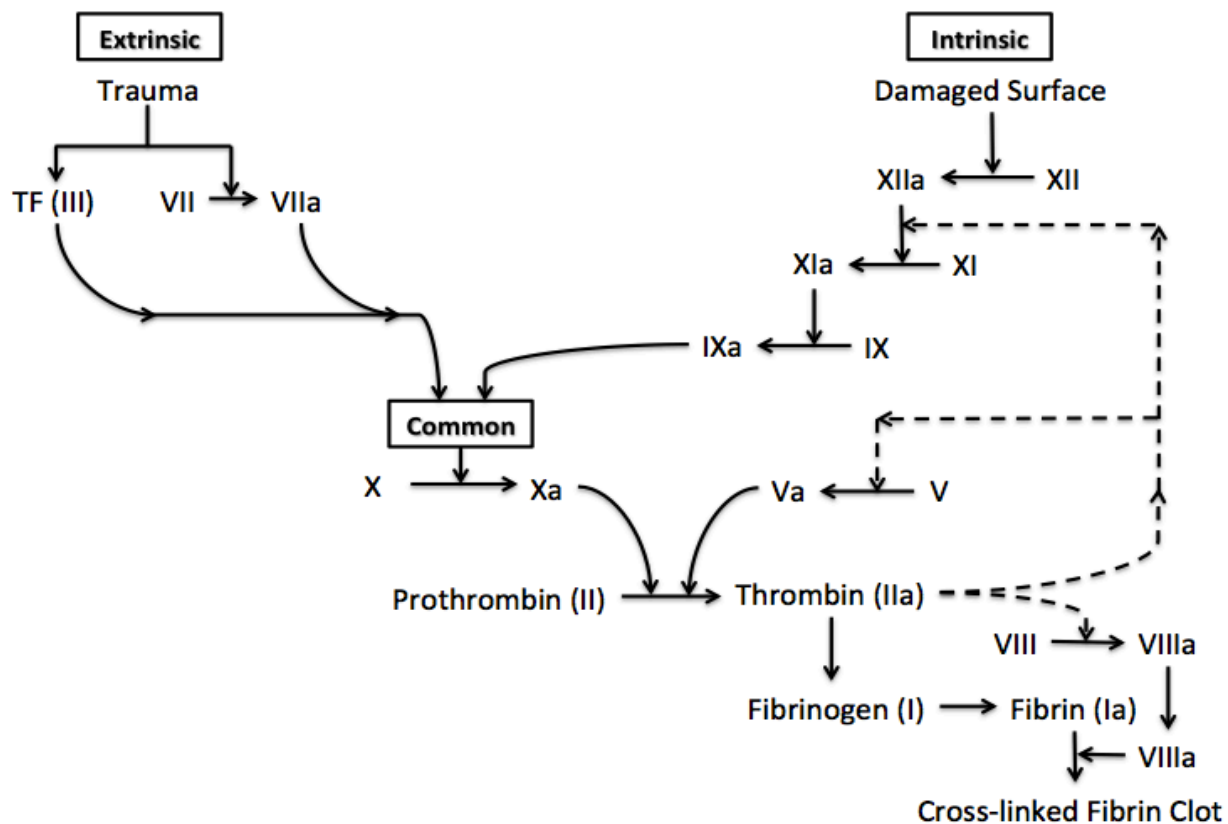


Figure A – The coagulation cascade and its respective pathways that lead to the formation of a cross-linked fibrin clot. Several proteins, also known as factors, work in conjunction to form and activate substrates to eventually halt the flow of blood through the wall of a damaged blood vessel. A solid arrow (\rightarrow) indicates direct conversion of one factor to

another and a dashed arrow (-->) indicates thrombin acting on other proteins in positive feedback fashion [37].

Blood Coagulation and Health

The ability of one's blood to clot, or the coagulation potential, has been directly related to various cardiovascular risk factors and to the onset of several adverse cardiovascular events [5]. An increase in the likelihood of thrombus formation, or thrombosis, can increase one's risk for CVD [2] or the incidence of several cardiovascular events including myocardial infarction, stroke, angina, and sudden cardiac death [4, 38-40]. While factor VII and factor VIII have been shown to be positively and independently associated with the incidence of thrombus formation [41]. Although fibrinogen has not been directly associated with thrombus formation, it is commonly identified as an independent risk factor for CVD [42]. While the coagulation cascade contains many proteins, these specific plasma proteins have been shown to consistently change with the onset of CVD [39, 41, 42]. An increased plasma concentration of these coagulation cascade enzymes (e.g., factor VIII and fibrinogen) leads to an increased potential for blood coagulation.

While an increased coagulation potential is associated with an increased risk for disease, a reduced coagulation potential has been related to an overall improvement in health [23]. Markers of blood coagulation have been studied extensively and related to outcomes of coronary disease in several cohorts and populations [4]. Higher plasma levels of factor VII activity have been related to cardiac death [43], while higher plasma levels of fibrinogen have been related to myocardial infarction and cardiac death [43-45]. Therefore, lower plasma levels of these proteins are associated with a reduced coagulation

potential. However, blood coagulation parameters are affected by several risk factors including: age, sex, smoking, alcohol, and obesity [31, 32, 46-48].

Several proteins that are important markers of blood coagulation (e.g., fibrinogen, factor VII, factor VIII) have been related to the incidence of CVD and other adverse outcomes through aging. Factor VII, factor VIII, and fibrinogen are associated with aging, as increased plasma levels have been observed in the elderly [31]. Likewise, Sugawara et al. determined that plasma levels of most coagulation factors are significantly increased with aging in sedentary men, although this tendency seems to disappear in regularly active men. They specifically stated that the most prominent increases in coagulation proteins were seen in fibrinogen, factor IX, and factor XIII [5]. Following regular physical conditioning, Van Den Burg and colleagues determined that factor VIII levels were most pronounced in young individuals while thrombin formation was most pronounced in older individuals [14]. A comparison study between the young and the elderly showed that the elderly possess an increased plasma concentration of coagulation proteins, which led to shorter clotting times and an increased coagulation potential. Specifically, the elderly experienced an increase in fibrinogen [49]. Many of the proteins that have been shown to be increased dependent upon age are risk factors for thrombotic disease [31] and a reason for further investigations. It is also important to note that genetic and environmental factors such as diet, smoking, exercise, and hormonal status modulate hemostatic parameters through aging [50].

Furthermore, an increased coagulation potential has been related to gender, specifically with regards to women, pregnancy, and oral contraceptives [46, 51, 52]. A study that used thromboelastography, a point-of-care monitor of whole blood coagulation,

found that women have higher clotting activity than men. In comparing men, non-pregnant women, and pregnant women, it was determined that women have an increased coagulation potential compared to men, and pregnant women experience the most increased coagulation potential [46]. Specifically, in pregnancy, a hypercoagulable state is developed because of an increase in several protein concentrations (i.e., factor II, factor VII, factor VIII, factor X, and factor XI) [51]. Another article shows that women experience increased concentrations of fibrinogen and factor VIII, which would explain sex differences in blood coagulation potential [53]. A few studies extended their investigations to show that like pregnancy, oral contraceptives have a similar effect on overall blood coagulation [52, 54]. Rosendaal and colleagues took an in-depth look at thrombosis and concluded that venous thrombosis in young women can commonly be attributed to use of oral contraceptives [52]. However, Winkler argued that not all oral contraceptives have the same outcome on overall markers of blood coagulation [54]. While the use of oral contraceptives is associated with altered plasma levels of several proteins of the coagulation system, not all populations respond the same and further research needs to be executed [54].

Although controllable, another major risk factor for increased coagulation potential is cigarette smoking. However, regular cigarette smoking has produced equivocal results with regards to blood coagulation. Some studies have correlated regular cigarette smoking with higher coagulation protein levels [55, 56] while others have shown no negative effects of regular cigarette smoking [47, 57]. It is postulated that the nicotine in cigarettes is what causes an increased coagulation potential because of the vasoconstrictive action of nicotine. However, the same authors argued that the act of deep breathing might induce a

similar degree of vasoconstriction [58]. Although vasoconstriction is an important initial stage of blood clotting, a change in protein levels will be the ultimate factor that alters blood coagulability. Mustard and Murphy conducted a study that showed no changes in the importance of blood coagulation in response to heavy smoking [47]. Likewise, another study found that following a meal, the protein rise in smokers was less than that of non-smokers, leading to a negligible difference in blood coagulation [57]. On the contrary, several studies found regular smoking to have a significant increase on coagulation potential. Billimoria et al. measured serum levels of many coagulation proteins as well as clotting times, and showed a shortened PT in smokers compared to non-smokers [56]. A more recent study suggested that elevated factor XIII levels due to smoking are associated with the pathogenesis of vascular disease. They also concluded that factor XIII activity is significantly related to fibrinogen levels [55].

Alcohol consumption has also been shown to affect coagulation potential. Light to moderate alcohol consumption (i.e., up to one drink per day for women and up to two drinks per day for men) has been associated with a reduced coagulation potential [48]. While the usage of recreational drugs is never advised, the hemostatic system may respond differently dependent upon the type of alcoholic beverage, sex, time of day, and during exercise [48, 55].

Obesity is a major risk factor for cardiovascular disease in both men and women [59, 60]. It is well known that obesity is correlated with an increase in several plasma proteins including von Willebrand factor, fibrinogen, factor VII, and factor VIII [32, 61]. The underlying mechanism for the increase in coagulation potential and obesity lies within the adipocyte itself. The adipocyte is able to produce plasminogen activator inhibitor-1,

which explains the high plasma levels of proteins [32]. The relationship between obesity and an increase in coagulation potential is well understood and known to promote a prothrombotic state, which increases one's likelihood of CVD [32]. Other factors, such as dietary lifestyle [61] and insulin sensitivity [32], have been shown to be associated with the prothrombotic state that obesity brings but hemostatic variables are the most well known.

A prothrombotic state is directly related to an increased risk for CVD and can be caused by several mechanisms, including: age, sex, smoking, alcohol, and obesity [31, 32, 46-48]. However, even if these mechanisms are not present, a prothrombotic state is still independently associated with adverse cardiovascular events [23]. Research has shown that an increased coagulation potential may be present in healthy individuals who do not otherwise have risk factors for cardiovascular disease [42]. A reduced coagulation potential has been related to an overall improvement in health [23], but several variables of the hemostatic system can be easily affected by slight changes to the body. Discussed below, blood coagulation may or may not be altered following exercise, whether it is an acute bout or a longer-term exercise training program.

Exercise Training and Blood Coagulation

Regular exercise training is inversely related to the incidence and risk of several adverse outcomes associated with CVD and other chronic diseases [62]. Despite the large body of evidence supporting the benefits of regular physical activity and the known dose-response relationship between activity and disease, there is no consensus regarding the required dose of exercise needed to promote desired physiological outcomes [28]. While regular physical activity promotes a cardioprotective state, the outcomes with regards to the hemostatic system remain controversial or unknown. The three main types of exercise

training that have been related to blood coagulation include: endurance training, resistance training, and HIIT.

Changes in APTT, PT, and concentrations of fibrinogen and factor VIII have been related to a significant change in overall coagulation potential [26]. Several studies use a cross sectional approach to compare blood coagulation potential related to different fitness or physical activity levels. Ferguson et al. compared three groups of individuals: marathoners, joggers, and sedentary individuals, and determined that there were no differences in APTT and PT between groups at rest and post exercise [12]. Subjects in this study all possessed significantly different VO_2 max values suggesting that coagulation and VO_2 are not correlated. Another study screened for physical activity level and found no significant differences in APTT between groups at rest [63]. Likewise, El-Sayed observed significant differences in VO_2 max in a mixed sample and found no changes in coagulation activity [64]. However, several cross sectional studies consistently observed an inverse relationship between regular physical activity and fibrinogen [15, 63, 65-68] and factor VIII activity levels [63, 69]. Furthermore, Rankinen et al. observed an inverse correlation between fibrinogen levels and VO_2 max [70]. The inability of these studies to establish cause and effect or eliminate other factors (e.g., genetic and environmental) is why longitudinal studies are preferred and also why more research is needed with regards to this topic and different modes of exercise training.

Endurance Training

While endurance training is the most commonly researched mode of exercise, it has produced the most equivocal results with regards to blood coagulation [26, 27]. APTT, which measures the time it takes to get through the intrinsic pathway, has been shown to

be prolonged with exercise training [10, 71], but some studies suggest a significant shortening in clotting times [14, 63]. Suzuki and colleagues demonstrated that four weeks of physical training suppressed blood coagulation via a prolongation in APTT [71]. This study observed 56 post-myocardial infarction patients (men and women) before and after one month of physical training. The moderate intensity, high volume training protocol consisted of cycling and walking for two times a day, six days a week. Likewise, Hilberg and associates showed that twelve weeks of endurance training had a significant influence on the prolongation of APTT at rest and following an acute exercise bout compared to before the training program [10]. The subjects in this study were healthy untrained men, aged 40-60 years old. These individuals walked, ran, or biked at 80-100% of their individual anaerobic threshold for 60 minutes three to four times a week.

On the contrary, a study using moderately active but untrained subjects found a significant shortening in APTT following a maximal exercise test both before and after twelve weeks of aerobic cycling [64]. Subjects exercised for 30 minutes, three days per week at an intensity of 70-80% of maximum heart rate. Another longitudinal study, with a similar training protocol, demonstrated that twelve weeks of aerobic conditioning also resulted in a shortening of APTT in sedentary men [14]. Although the measures for this study were taken at rest, it produced similar results to the study mentioned above. Van den Burg et al. observed sedentary men of all ages who exercised twice a week for one hour at 60-70% of VO_2 max.

Another measure of clotting time, PT, or the time it takes to get through the extrinsic pathway, has produced inconsistent results as well [10, 11, 71]. Suzuki's study of post-myocardial infarction patients also demonstrated that exercise training did not affect PT

[71]. Similarly, the study by Hilberg et al., described above, showed that PT was not affected following 12 weeks of aerobic exercise training pre and post an acute exercise bout [10]. Lockard et al. conducted a study that suggests that moderate endurance training has a beneficial effect of reducing coagulation potential in the common pathway. Sedentary men and women underwent aerobic exercise training three days a week for six months at exercise duration and intensity of 40 min at 70% heart rate reserve. Although not measuring the extrinsic pathway directly, they concluded that the training induced changes of clotting potential are independent upon lipid profile, body composition, and aerobic capacity [11].

Plasma fibrinogen levels are commonly measured when it comes to coagulation potential [26], but yield conflicting results. Ernst et al. conducted a study on sedentary males and showed that nine weeks of endurance training reduced fibrinogen levels compared to the control group [72]. Another study, examining post-bypass patients, showed that fibrinogen levels decreased significantly with aerobic training compared to power training or no formal exercise training following three and six months [73]. Likewise, Stratton and associates observed a decrease in fibrinogen levels following six months of intense endurance training [74].

A study in 1997 observed the opposite changes in fibrinogen. Schuit et al. found an increase in plasma fibrinogen following exercise training in the elderly [75]. This study consisted of 829 independently living elderly men and women, aged 60-80, exercising on a bicycle at 50-70% of maximal heart rate. The increase in fibrinogen levels may be attributed to the elderly cohort in this study and the delayed mechanisms in response to extensive exercise training. A few other studies [76, 77] also found an increase in

fibrinogen levels in trained individuals several days after an exercise stimulus. Montgomery et al. observed 165 male army recruits before and after their 10-week basic training camp [76]. Although the protocol was only described as vigorous physical fitness, the unique program may explain the increase in fibrinogen. Ponjee et al. also observed an increase in fibrinogen with a training program that involved sedentary men and women. These subjects trained four times a week at increasing intensities for nine months, to prepare for a half-marathon race [77].

While several studies indicate an increase or decrease in plasma fibrinogen levels, some studies suggest that fibrinogen levels do not change in response to regular endurance training. Different populations of males who have undergone endurance training demonstrated no change in plasma fibrinogen [78, 79]. Rauramaa and colleagues observed 140 middle aged men over a three year long, low intensity exercise intervention, which may explain their findings [78]. Similarly, Tisi observed men and women over a yearlong period of low intensity exercise, involving walking and leg exercises [79].

Studies regarding factor VIII activity and factor VIII antigen levels also have produced inconsistent results following endurance training. Investigations pertaining to sedentary individuals show no change in factor VIII following endurance training [14, 80, 81]. However, a previously mentioned study conducted by Suzuki and colleagues showed that four weeks of endurance training lowered resting levels of factor VIII activity and factor VIII antigen [71]. These results may be explained by the nature of their population who were all post-myocardial infarction. Thus, longitudinal investigations are conclusive with respect to the influence of exercise training on blood concentrations of factor VIII.

Resistance Training

Few studies have been published with regards to resistance training and blood clotting and, to my knowledge, there are no published longitudinal studies assessing the effect of strength training on coagulation potential. Cross-sectional analyses of resistance training on blood clotting parameters have yielded inconsistent results. Nascimento and colleagues published an informative review on the interactions between hemostasis and resistance training in 2012 [82]. While most of the studies presented were acute based resistance training studies, a few of them compared resistance trained to untrained individuals at rest. A study conducted in 2005 showed no significant differences between resistance trained and untrained individuals in any parameters measured, including fibrinogen levels [83]. The same group looked at platelet activation and function the following year in resistance trained and untrained individuals and found no significant differences between the two groups [84]. Likewise, El-Sayed observed no significant differences in factor VIII activity over three resistance training programs at rest [85]. El-Sayed's study consisted of nine common exercises straining major muscle groups in both the upper and lower body. A more recent study suggests that resistance trained individuals exhibit a lower capacity to form a clot via APTT [13]. This group also demonstrated no changes in fibrinogen and several other hemostatic parameters between resistance trained and untrained individuals. Although fibrinogen levels were unchanged, this new insight, in contrary to findings in previous studies, suggests that habitual resistance training may be a promising mode of exercise to enhance health outcomes and reduce the risk of CVD [13].

Acute, High Intensity Exercise

The literature is consistent regarding the effects of an acute bout of exercise on blood clotting potential. A single Wingate anaerobic exercise test led to a significant

increase in coagulation parameters, including APTT and factor VIII [86]. While this induces an increased coagulation potential, no investigations have been done with regard to this type of exercise as a training program. As shown in this study, blood coagulation parameters respond to acute bouts of this type of training and, because of the theory that exercise training reduces coagulation potential HIIT may be a promising substitute and beneficial mode of exercise training.

HIIT

It has been shown that regular exercise training is beneficial to overall health [3, 62, 87]. The American College of Sports Medicine (ACSM) recommends exercising at a moderate intensity for at least 150 minutes per week [88]. This can be met through 30-60 minutes of moderate-intensity exercise five days per week or 20-60 minutes of vigorous-intensity exercise three days per week. Although many individuals struggle to meet this recommendation because of a lack of time [89, 90], they may still receive some benefits from normal daily activities [88]. It has been shown that lack of time is a recurring theme with regards to exercise referrals and compliance [91]. A few investigations analyzed HIIT, and showed a reduced time component in comparison to endurance training [92, 93]. Meanwhile, it has been reported that participants perceive HIIT to be more enjoyable than continuous, moderate intensity exercise [94]. Furthermore, because HIIT is a newer mode of exercise training, ACSM's standard exercise recommendations do not incorporate it and there is a significant gap in the literature with regards to HIIT and the health benefits associated with this type of exercise training.

HIIT is characterized by short, intermittent bouts of vigorous physical activity, interspersed with brief periods of passive or low-intensity active recovery. The most

common HIIT protocols use 5-30 seconds of high intensity cycling or sprinting, followed by a brief recovery, repeated 3-6 times each session. Recent studies with regard to HIIT have produced consistent results that indicate this relatively novel mode of exercise training is beneficial to the cardiovascular system [19, 21, 92, 95-98], the respiratory system [22, 99, 100], and overall health [93, 101-103] in both healthy and diseased populations [17-21, 104]. A few studies have even suggested that HIIT may be an effective replacement for endurance training [92, 93, 98]. While blood coagulation has not yet been specifically related to HIIT, this type of training has been shown to induce beneficial improvements in endothelial function, resting blood pressure, and left ventricular morphology [17, 20, 22], each of which are related to the hemostatic system [23-25].

Endothelial dysfunction, which is used as an independent prognostic marker for adverse cardiovascular events [105], may be improved following HIIT. Wisloff and colleagues conducted a study to observe the enzymatic responses of the endothelium in response to HIIT [20]. This study examined the elderly population (aged 75 ± 11.1 years) and focused specifically on cardiac patients. They showed that three weeks of HIIT improved nitric oxide mediated endothelial function, and concluded that this improvement is dependent on the intensity of the exercise [20]. The exercise training included four 4-minute intervals with an exercise intensity that induced heavy breathing for the subjects. Likewise, scientists at McMaster University observed that 6 weeks of HIIT improves endothelial function [98]. This study observed significant changes in young, healthy men and women, and compared HIIT to endurance training at a matched work level. The HIIT protocol used in this study involved repeated Wingate tests with 4.5 minutes of recovery between each bout. A few other studies observed no significant

improvements in endothelial function with severe physical training [106, 107]. The discrepant findings of these studies may be due to the different intensities of exercise. Bergholm et al. and Goto et al. observed light, moderate, and high intensity exercise and observed no endothelial improvements following moderate intensity exercise but observed improvements following high intensity exercise [106, 107]. The goal of these studies was to elucidate where the endothelial function training adaptation occurred with respect to different exercise intensities.

Systolic and diastolic blood pressures have also been shown to be prognostic markers of CVD [108] and are independent risk factors for various cardiovascular events [88]. While some articles have shown no improvements in blood pressure readings following HIIT [98, 109], one study disagreed [22]. Whyte et al. showed significant reductions in systolic blood pressure following two weeks of Wingate-based HIIT [22]. The differences between these studies lie within the subject population. Studies that showed no significant changes in blood pressure consisted of subjects who were healthy, but sedentary at the beginning of the intervention. On the other hand, the subjects used in the Whyte et al. paper were classified as overweight or obese before beginning the HIIT. These data suggests that healthy subjects may not see an altered blood pressure following this type of training, but overweight or obese subjects may see significant improvements.

Left ventricular remodeling can be seen following three weeks of aerobic interval training [20]. This is shown via levels of plasma proBNP, a marker of hypertrophy and severity of heart failure. The subjects in this study were post-myocardial infarction. Along with lower levels of proBNP and improved ventricular morphology, Wisloff and colleagues observed reduced left ventricle end-diastolic and end-systolic volumes [20]. Mentioned

previously, the Wisloff paper is unique because they observed subjects from an elderly population who have previously experienced an adverse cardiac event. This study suggests that HIIT may be an effective replacement for endurance training, even in cardiac patients.

Finally, a few studies observed overall quality of life following HIIT and showed significant improvements [17, 20, 110]. The similarities between these three studies lie within the subject population as each cohort of subjects had previously experienced a cardiac event. These measurements mirror and provide further support for the aforementioned physiological improvements. These studies suggest that pronounced physiological adaptations provided an improved exercise capacity and an overall improvement in quality of life.

Summary

There is an indirect relationship between the hemostatic system and the incidence and risk for CVD [5]. The hemostatic system is affected by several physiological mechanisms including age, sex, smoking, alcohol, and obesity [26, 27, 31-33, 48] as well as exercise and exercise training [26, 111]. Literature suggests that hemostatic system and overall health will benefit from exercise training, especially endurance training. However, the literature is still unclear and inconsistent at times with regards to this mode of exercise training [26, 111]. A novel mode of exercise training, HIIT, has not yet been examined with regards to the hemostatic system. It is hypothesized that since this mode of exercise has shown endothelial, blood pressure, and left ventricular morphology improvements [17, 20, 22], HIIT will also show improvements within the hemostatic system, especially blood coagulation. The overall goal of this study is to prove that HIIT is a cardioprotective mode of exercise training.

CHAPTER III

METHODOLOGY

The purpose of this study was to examine the hemostatic adaptations in healthy adult men following four and eight weeks in a high-intensity interval training (HIIT) intervention. All procedures were reviewed and approved by the Institutional Review Board at Ball State University.

Subjects

Twenty-one apparently healthy men (age: 25 ± 1 yr.; BMI: 26.7 ± 6.2 kg/m²) voluntarily participated in the present study. Prior to participating in the study, all participants provided written informed consent and completed a brief health history questionnaire that was used to screen for conditions that may compromise the safety of the subject or the integrity of the data during participation in the study. Subjects were apparently healthy, non-smoking, sedentary men between 18 and 45 years old. Subjects weighed at least 50 kg. In this study, sedentary was defined as participating in aerobic

physical activity fewer than two days a week and less than 30 minutes a day within the past three months. Subjects were free from known cardiovascular or metabolic disease and excluded from the study if they possessed two or more risk factors or were considered “high risk” according to the American College of Sports Medicine [88].

Recruitment

Subjects for this study were recruited from the Ball State community through e-mail, flyers, and word of mouth. Subjects were required to attend at least 20 of the 24 exercise training sessions and complete the intervention within a ten-week period or they were excluded from analysis. A baseline blood sample was not collected from one subject due to technical complications. Two subjects dropped out following four weeks for personal reasons and one subject dropped after one training session due to personal reasons. Furthermore, the lack of hematocrit values from two subjects disallowed for plasma volume corrections and no fibrinogen calculations at 4w and 8w.

Experimental Design

Subjects were asked to report to the Integrative Exercise Physiology Laboratory (IEPL) for data collection on three separate occasions, and for exercise training on twenty-four separate occasions. Each data collection session involved a blood draw from an antecubital vein, and the exercise training sessions involved three to six bouts of a standardized Wingate test. The total time commitment for data collection was less than three hours, and exercise training required approximately twelve hours over eight weeks (total time commitment of 15 hours).

Data Collection Protocol:

Data collection took place within one week prior to beginning the training program and one week after the program. To assess the time course of putative training adaptations, an additional data collection took place after the fourth week of training. Subjects were asked to abstain from exercise for 24 hours prior to data collection, as well as alcohol, caffeine, and food or drink (except water) for 12 hours prior. The data collection period began with 15 minutes of semi-recumbent rest, after which a 10-ml blood sample was taken from an antecubital vein using clean venipuncture with minimal stasis. Following the blood draw, subjects were instructed to move to a plinth where they experienced 10 minutes of supine rest. At this point, two resting brachial blood pressures were obtained via auscultation using an aneroid sphygmomanometer, calibrated to a wall mounted mercury column. Resting heart rate was also calculated following 10 minutes of supine rest using an ECG tracing analyzed by the SphygmoCor (Itasca, IL). Other vascular measures were then collected and can be found in a separate manuscript.

Exercise Training:

The subjects reported to the IEPL for exercise training three days/week for eight consecutive weeks. Subjects weight was recorded at the beginning of each week in order to set the proper intensity on the Monark cycle ergometer. Prior to each training session the subjects warmed up with five minutes of cycling at 50 watts. Each training bout consisted of 30 seconds of high-intensity cycling followed by 4.5 minutes of active recovery. During the first two weeks, training sessions included three bouts of cycling. With each successive two-week period, an additional bout was added to the training session, so that four bouts were performed per session in weeks 3-4, five bouts in weeks 5-6, and six bouts in weeks 7-8. The training bout was modeled after a standard Wingate anaerobic test [112]. Briefly,

the subject pedaled as fast as possible with the resistance on the flywheel set to 7.5% of body weight in kg. Active recovery between bouts included walking or cycling at a self-selected, low intensity.

Blood Sampling/Processing:

Blood samples were immediately centrifuged for 20 min at 2,750xg at 4°C to obtain platelet-poor plasma. Plasma was separated from the red blood cells and spun again for 5 min at 2,750xg at 4°C to assure platelet-poor plasma. Plasma aliquots were frozen and stored at -20°C until assayed. Clotting time measures (activated partial thromboplastin time [APTT] and prothrombin time [PT]) and plasma concentrations of fibrinogen were assessed by a coagulometer (Start4®, Diagnostica Stago; Parsippany NJ) according to manufacturer specifications. Briefly, calcium chloride was added to plasma samples to initiate a clotting reaction. The interrupted motion of a magnetic stir implement due to fibrin formation was monitored to determine coagulation time.

Statistical Analysis

All statistical analyses were conducted using SPSS for Windows (version 22, SPSS, Chicago, IL). Subject characteristics (age, height) are depicted using descriptive statistics. Resting measures of body mass index, resting blood pressure, resting heart rate, and blood clotting parameters were analyzed at baseline, 4-week, and 8-week using repeated measures ANOVA with time points as the within-subjects variable. Further, exercise training variables, mean power (MP), peak power (PP), and fatigue index (FI), were averaged for each week and analyzed using a repeated measures ANOVA with time points as the within-subjects variable. All data was normally distributed according to the Shapiro-Wilk test of normality. Greenhouse-Geisser adjustments were used when Mauchly's test of

sphericity was significant ($p < 0.05$). Significant statistical analyses were followed up using the Fisher's LSD test. The following variables were included in the analyses: MP (W); PP (W); FI (%); APTT (sec); PT (sec); and plasma concentrations of fibrinogen (mg/dL). Four-week and eight-week fibrinogen concentrations were corrected for plasma volume changes using the VanBeaumont equation [113]. Statistical significance was set at $\alpha < 0.05$.

CHAPTER IV

RESEARCH MANUSCRIPT

Journal Format: Medicine & Science in Sports & Exercise

Title: Hemostatic Adaptations Following a High-Intensity Interval Training Intervention in Healthy Men

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Running Title: Hemostasis and HIIT

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Conflict of Interest: None

ABSTRACT

High intensity interval training (HIIT) is a novel mode of exercise training that has been shown to improve several components of health in various healthy and diseased populations. **Purpose:** The purpose of the present study was to examine hemostatic adaptations in healthy adult men following four and eight weeks of a HIIT intervention. **Methods:** Twenty-one healthy but sedentary men (age: 25 ± 5 yrs; BMI: 26.7 ± 6.2 kg/m²) volunteered to participate in the present study. Subjects completed eight weeks of HIIT that included three to six 'all out' Wingate tests three days/week. Overall blood coagulation was assessed at baseline, following four weeks, and following eight weeks by clotting times of activated partial thromboplastin time (APPT) and prothrombin time (PT), and plasma concentration of fibrinogen. Plasma was obtained from whole blood samples taken at rest. A repeated measures ANOVA was used to compare the overall coagulation potential. Significance was set to $p < 0.05$. **Results:** There were no significant differences between resting heart rate, resting blood pressure, APTT (baseline: 43.0 ± 5.4 ; 4w: 42.7 ± 5.1 ; 8w: 44.2 ± 6.2), and PT (baseline: 13.0 ± 0.9 ; 4w: 12.9 ± 0.6 ; 8w: 13.1 ± 0.8), after 4w or 8w of HIIT. Fibrinogen concentrations significantly decreased from baseline to 4w ($p < 0.05$) and significantly increased from 4w to 8w ($p < 0.05$). **Conclusion:** Although beneficial fibrinogen changes were seen following four weeks of training, these findings were reversed after eight weeks. These observations suggest that HIIT may elicit improvements in coagulation potential after four weeks, but continued training may lead to elevated coagulation and/or inflammation via fibrinogen, which is recognized as a key regulator of inflammation and disease.

Key Words: Hemostasis, Thrombosis, Coagulation, HIIT, Interval, Training

INTRODUCTION

Cardiovascular disease (CVD) remains the most common cause of death in the world today (27). CVD or a CVD-related event accounted for 24.2% of the total deaths in 2010 (27). It is well known that exercising regularly will help prevent CVD and several CVD complications (4, 17). The majority of cardiovascular events, including approximately 80% of acute myocardial infarctions, are initiated by a blood clot, or thrombus, that occludes arterial blood flow (3). The ability of one's blood to clot, or the coagulation potential, has been directly related to the onset of CVD as a thrombus may occur (34). Such thrombotic events that cause an ischemic event are directly related to the balance of the hemostatic system (34). The prediction of coronary risk can be determined by several factors (e.g., FVIII and fibrinogen) as these factors may promote a procoagulant state of the hemostatic system (4). It is theorized that exercise training reduces one's overall coagulation potential, as this is one of the reasons that regular exercise is cardioprotective (43).

Several previously published studies suggest ACSM's standard exercise recommendation (2) inconsistently influences coagulation potential (10, 13, 18, 22). Traditional aerobic exercise training may result in increased (15, 21, 37), decreased (7, 18, 22) or unchanged coagulation potential (11). These equivocal results related to exercise training support the need for further investigations of training regimens that may deviate from traditional recommendations. High-intensity interval training (HIIT) involves short, intermittent bouts of vigorous physical activity, interspersed with brief periods of passive or low-intensity active recovery. HIIT has been shown to improve markers of aerobic physical fitness in various healthy and diseased populations (23, 25, 26, 40, 42). Also, HIIT has been shown to improve endothelial function, resting blood pressure, and left

ventricular morphology (23, 41, 42), each of which is related to coagulation potential (16, 29, 38).

Previous research indicates that coagulation responses to acute exercise are intensity dependent (43), leading to the speculation that the moderate intensities used in traditional exercise training may not stimulate the hemostatic system in such a way that would lead to beneficial changes in coagulation potential. Despite the large body of evidence supporting the benefits of regular exercise and the known dose-response relationship between activity and disease, there is no consensus regarding the required dose of exercise needed to promote desired physiological adaptations (12, 20). Blood clotting adaptations following a HIIT regimen are unknown. Thus, the purpose of this study was to evaluate the effects of four and eight weeks of HIIT on measures of blood coagulation potential. It was hypothesized that following four and eight weeks of HIIT subjects would experience a reduced coagulation potential with regards to clotting times of activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen concentration.

METHODOLOGY

All procedures were reviewed and approved by the Institutional Review Board at Ball State University.

Subjects

Twenty-one apparently healthy men (age: 25 ± 1 yr.; BMI: 26.7 ± 6.2 kg/m²) voluntarily participated in the present study. Prior to participating in the study, all participants provided written informed consent and completed a brief health history questionnaire that was used to screen for conditions that may compromise the safety of the

subject or the integrity of the data during participation in the study. Subjects were apparently healthy, non-smoking, sedentary men between 18 and 45 years old. In this study, sedentary was defined as participating in aerobic physical activity fewer than two days a week and less than 30 minutes a day within the past three months. Subjects were free from known cardiovascular or metabolic disease and excluded from the study if they possessed two or more risk factors or were considered “high risk” according to the American College of Sports Medicine (2).

Recruitment

Subjects for this study were recruited from the Ball State community through e-mail, flyers, and word of mouth. Subjects were required to attend at least 20 of the 24 exercise training sessions and complete the intervention within a ten-week period or they were excluded from analysis. A baseline blood sample was not collected from one subject due to technical complications. Two subjects dropped out following four weeks for personal reasons and one subject dropped after one training session due to personal reasons. Furthermore, the lack of hematocrit values from two subjects disallowed for plasma volume corrections and no fibrinogen calculations at 4w and 8w.

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three hours, and exercise training required approximately twelve hours over eight weeks (total time commitment of 15 hours).

Data Collection Protocol: Data collection took place within one week prior to beginning the training program and one week after the program. To assess the time course of putative training adaptations, an additional data collection took place after the fourth week of training. Subjects were asked to abstain from exercise for 24 hours prior to data collection, as well as alcohol, caffeine, and food or drink (except water) for 12 hours prior. The data collection period began with 15 minutes of semi-recumbent rest, after which a 10-ml blood sample was taken from an antecubital vein using clean venipuncture with minimal stasis. Following the blood draw, subjects were instructed to move to a plinth where they experienced 10 minutes of supine rest. At this point, two resting brachial blood pressures were obtained via auscultation using an aneroid sphygmomanometer, calibrated to a wall mounted mercury column. Resting heart rate (RHR) was also calculated following 10 minutes of supine rest using an ECG tracing analyzed by the SphygmoCor (Itasca, IL). Other vascular measures were then collected and can be found in a separate manuscript.

Exercise Training: The subjects reported to the IEPL for exercise training three days/week for eight consecutive weeks. Subjects weight was recorded at the beginning of each week in order to set the proper intensity on the Monark cycle ergometer. Prior to each training session the subjects warmed up with five minutes of cycling at 50 watts. Each training bout consisted of 30 seconds of high-intensity cycling followed by 4.5 minutes of active recovery. During the first two weeks, training sessions included three bouts of cycling. With each successive two-week period, an additional bout was added to the training session, so that four bouts were performed per session in weeks 3-4, five bouts in weeks 5-

6, and six bouts in weeks 7-8. The training bout was modeled after a standard Wingate anaerobic test (5). Briefly, the subject pedaled as fast as possible with the resistance on the flywheel set to 7.5% of body weight in kg. Active recovery between bouts included walking or cycling at a self-selected, low intensity.

Blood Sampling/Processing: Blood samples were immediately centrifuged for 20 min at 2,750 \times g at 4°C to obtain platelet-poor plasma. Plasma was separated from the red blood cells and spun again for 5 min at 2,750 \times g at 4°C to assure platelet-poor plasma. Plasma aliquots were frozen and stored at -20°C until assayed. Clotting time measures (APTT and PT) and plasma concentrations of fibrinogen were assessed by a coagulometer (Start4®, Diagnostica Stago; Parsippany, NJ) according to manufacturer specifications. Briefly, calcium chloride was added to plasma samples to initiate a clotting reaction. The interrupted motion of a magnetic stir implement due to fibrin formation was monitored to determine coagulation time.

Statistical Analysis

All statistical analyses were conducted using SPSS for Windows (version 22, SPSS, Chicago, IL). Subject characteristics (age, height) are depicted using descriptive statistics. Resting measures of body mass index (BMI), resting blood pressure, RHR, and blood clotting parameters were analyzed at baseline, 4-week, and 8-week using repeated measures ANOVA with time points as the within-subjects variable. Further, exercise training variables, mean power (MP), peak power (PP), and fatigue index (FI), were averaged for each week and analyzed using a repeated measures ANOVA with time points as the within-subjects variable. All data was normally distributed according to the Shapiro-Wilk test of normality. Greenhouse-Geisser adjustments were used when Mauchly's test of

sphericity was significant ($p < 0.05$). Significant statistical analyses were followed up using the Fisher's LSD test. The following variables were included in the analyses: MP (W); PP (W); FI (%); APTT (sec); PT (sec); and plasma concentrations of fibrinogen (mg/dL). Four-week and eight-week fibrinogen concentrations were corrected for plasma volume changes using the VanBeaumont equation (36). Statistical significance was set at $\alpha < 0.05$.

RESULTS

Subjects

All results are expressed at means \pm SD. Twenty-one men volunteered for this study. Subjects were 25 ± 5 yrs old and 178.2 ± 10.0 cm tall. Demographic information for each time point (baseline, 4w, and 8w) is presented in Table 1. There were no significant differences between time points for age, height, weight, BMI, RHR, resting systolic blood pressure (SBP), and resting diastolic blood pressure (DBP).

Exercise Training

Weekly averages of PP, MP, and FI decreased significantly over the eight-week training period ($p < 0.05$). MP and PP weekly averages can be seen in Table 2. FI weekly averages can be seen in Figure 1.

Blood Coagulation

Blood clotting measures of APTT and PT were unchanged between the three time points and can be seen in Table 1. Baseline, 4w, and 8w values for the INR of PT were 0.79, 0.78, and 0.78, respectively, and not significantly different between time points. When the plasma was analyzed for fibrinogen concentrations, significant differences were evident between baseline and 4w measures ($p = 0.037$) and between 4w and 8w measures ($p =$

0.024). However, there were no significant differences between plasma concentrations of fibrinogen between baseline and 8w. Fibrinogen values are presented in Figure 2.

DISCUSSION

The major finding was that fibrinogen levels were significantly lower after 4w of HIIT compared to baseline, and significantly increasing over the remaining weeks of training, essentially returning to baseline levels. The significant decrease in fibrinogen from baseline to 4w supports our hypothesis that HIIT would cause a reduction in coagulation potential. Previous research indicates that fibrinogen levels are decreased following intense physical training (13, 33, 44). However, the rise in fibrinogen after the eighth week of training (back to baseline levels) is not supported by our hypothesis. While this significant increase in fibrinogen levels was unexpected, it has previously been seen in the literature (24, 30, 32). The increase in fibrinogen levels may be explained by the substantial increase in volume of exercise training that occurred in the latter four weeks of training compared to the first four weeks of training.

Several studies have examined plasma fibrinogen concentrations following intense physical training programs and a few studies showed fibrinogen levels to increase (24, 30). Ponjee et al. conducted a study in 1993 that showed similar results to our study (30). While the training regimen was unique in the aspect that the subjects were training for a half-marathon race, they showed an initial decrease in fibrinogen levels followed by a significant increase in fibrinogen levels that mirrored the increase in training volume and intensity. Sedentary men and women trained at increasing intensities and volumes for 9-months in preparation for a race via long-distance running, running at high speeds, and interval training. Similarly, another study showed an increase in fibrinogen in male army

recruits following 10 weeks of basic training (24). Although not specified in depth in the paper, one may expect the exercise protocol of basic training camp to be of extreme vigorous intensity and of high volume. Combining these results and the results of the present study, it is suggested that fibrinogen levels may decrease following general exercise prescription but may increase with a high intensity and/or volume of exercise training.

The observed changes in fibrinogen levels in the present study may be related to the fact that fibrinogen is an acute phase reactant protein. This indicates that plasma concentrations of fibrinogen will increase or decrease parallel to inflammation. Furthermore, previous research has demonstrated that intense physical training exacerbates inflammation (28). In pathological conditions, such as injury or diseases associated with vascular disruption, fibrinogen levels have been shown to increase several fold (1). While its role in the coagulation cascade is well established, a growing body of evidence implicates fibrinogen concentration as a key regulator of inflammation (9). Davalos & Akassoglou discuss how our understanding of fibrinogen is evolving from a building block of blood clots to an essential signaling molecule with a broad spectrum of functions, including extensive inflammation, eventually leading to life or death (9). Fibrinogen signaling is becoming more and more evident through its binding sites that are non-overlapping with those in the coagulation cascade (9). With the perspective that fibrinogen levels are related to inflammation, our data suggest that the first four weeks of training in this study did not cause chronic inflammation and therefore significantly reduced fibrinogen levels. This is comparable to what is seen in the literature with regards to traditional, aerobic exercise training (13, 44). However, when the volume of exercise

was ramped up (latter four weeks), it is theorized that chronic inflammation was stimulated, leading to a significant rise in fibrinogen levels through its several binding sites as it acts as a mediator of inflammatory disease.

While the rise in fibrinogen levels observed in the final four weeks of this study is still important with regards to thrombosis, it is postulated that this adaptation did not directly affect the coagulation cascade, a hypothesis that is supported by the results of the clotting time analyses. Unlike fibrinogen, no significant differences were found with regard to APTT between baseline, 4w, and 8w. Previously published articles showed APTT either increases (18, 35) or decreases (37, 39) with exercise training. With the significant changes in fibrinogen levels, one may expect to see APTT levels change as well because fibrinogen is a part of the coagulation cascade's intrinsic pathway, which is evaluated during an APTT test. However, a change in fibrinogen levels does not always affect the coagulation cascade. While fibrinogen levels increased in our subjects, it may not have actually affected coagulation activity, as APTT's did not change over the entirety of the study.

Similarly to APTT, no significant difference was found in PT between baseline, 4w, and 8w. However, contrary to the measure of APTT, a PT test does not assess the portion of the coagulation cascade in which fibrinogen plays a role. The fact that PT did not change throughout the training program is shown in the literature and confirmed with this study. Several investigations in the past have analyzed PT's and shown that exercise training does not affect it (15, 19, 35, 39). These studies encompassed different modes and different volumes of exercise as well as different populations of subjects. This finding, which is

comparable to what is seen in the literature, suggests that the extrinsic pathway is not a modulator in the coagulation changes associated with exercise training.

A limitation of this study was our subject population of college-aged men. Coagulation parameters differ significantly based on the population of the cohort and our subject population may not be generalizable to the other populations. It is thought that clinical and cardiac populations that may already have an elevated inflammation and/or coagulation potential might experience something different during HIIT. Another limitation of this study lies within the fact that we did not objectively measure a training adaptation. While it is believed that a training adaptation occurred, no improvements were shown via a Wingate test. Therefore, future research may want to measure training adaptations via a single Wingate test or VO_2 max test before and after the training program. Finally, the lack of a non-exercise or endurance training control group may have limited our ability to observe significant adaptations to HIIT.

Future research should focus on the goal to elucidate the volume of HIIT required to elicit beneficial improvements in blood clotting parameters. Specifically, it is important to find the volume and intensity of an exercise training program that yields the most beneficial values with regards to fibrinogen. Careful investigations need to be conducted with regards to the putative time course of adaptations and exercise intensity/volume. Several studies used six weeks of HIIT and showed positive changes (6, 31), but our eight week study did not concur. It is also suggested that future investigations extend HIIT into 10, 12, and more weeks to analyze the occurring adaptations. Furthermore, future research should explore inflammation in response to HIIT including other markers of

inflammation such as interleukin-6, C-reactive protein, interleukin-1, and/or adhesion molecules.

CONCLUSION

In conclusion, the present study indicates that a four-week HIIT program may reduce fibrinogen levels, but an additional four weeks of HIIT brought fibrinogen levels back to baseline values. It is unclear if continued HIIT would provoke further fibrinogen increases. This study elicited no change in APTT and PT values following four and eight weeks of HIIT. It is believed that blood clotting potential wasn't affected and that fibrinogen changes were due to an inflammatory response. While fibrinogen is an important marker of blood clotting in the coagulation cascade, it is becoming a more important mediator of inflammatory disease (9). Increased fibrinogen levels are commonly found in cardiac patients, and are an important risk factor for stroke (8, 14). This suggests that, because of HIIT's ability to induce inflammation, HIIT may be dangerous for those with increased levels of fibrinogen and should be avoided by subjects at risk. Results of this study may be important for medical and exercise professionals who work with individuals with elevated inflammation and high thrombotic risk.

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TABLE 1**Table 1.** Resting Measures and Blood Clotting Times

	Baseline	4w	8w
Weight (kg)	85.2 ± 21.1	85.8 ± 21.2	86.2 ± 21.1
BMI (kg/m²)	26.7 ± 6.2	26.9 ± 6.3	26.9 ± 6.3
RHR (bpm)	56.7 ± 11.2	55.4 ± 7.7	54.7 ± 9.0
SBP (mmHg)	111.2 ± 10.9	111.4 ± 12.6	109.3 ± 10.9
DBP (mmHg)	67.8 ± 8.8	69.4 ± 11.3	68.3 ± 8.4
APTT (sec)	43.0 ± 5.4	42.7 ± 5.1	44.2 ± 6.2
PT (sec)	13.0 ± 0.9	12.9 ± 0.6	13.1 ± 0.8

Resting measures and measures of blood clotting times are presented as mean ± SD (n = 18). No significant differences were seen between any of the time points (p > 0.05).

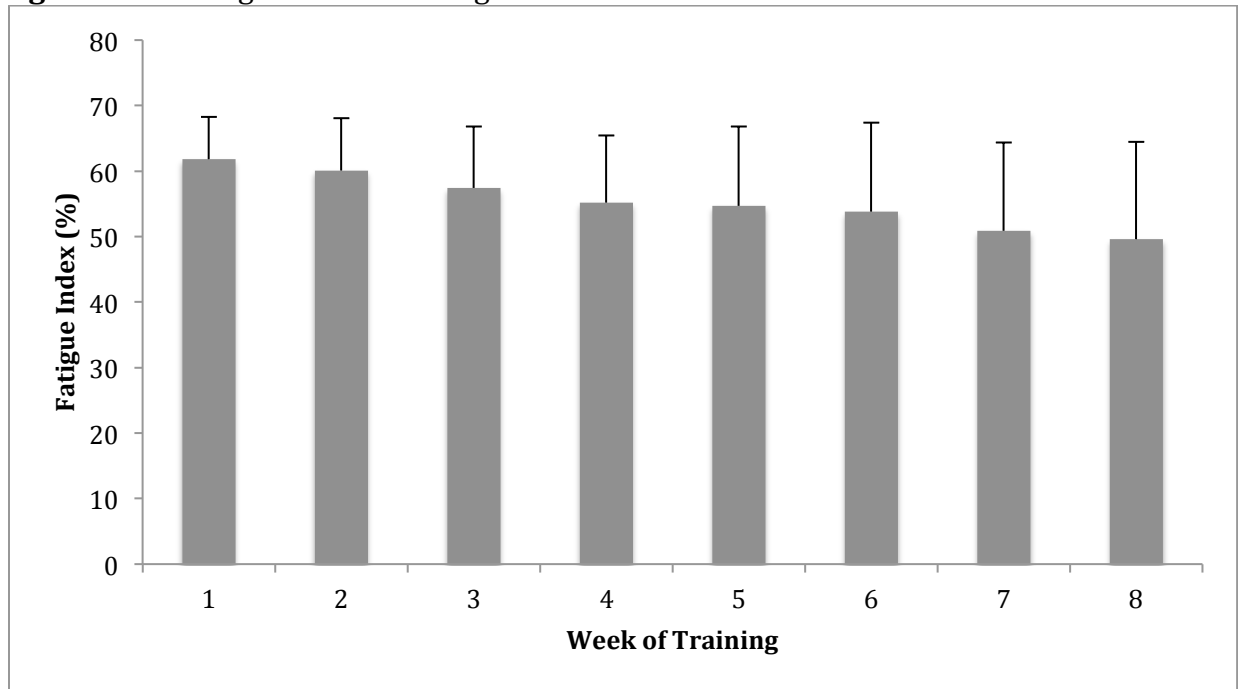
TABLE 2**Table 2.** Training Stimulus of Mean Power and Peak Power

	MP (W)	PP (W)
Week 1	511.7 ± 132.0	749.8 ± 205.4
Week 2	519.6 ± 132.9	753.2 ± 212.0
Week 3	512.4 ± 123.5	720.3 ± 182.2
Week 4	514.0 ± 121.4	712.2 ± 183.4
Week 5	497.5 ± 112.7	694.0 ± 183.8
Week 6	505.0 ± 114.2	708.1 ± 200.0
Week 7	493.9 ± 112.6	683.2 ± 197.2
Week 8	490.9 ± 119.6	665.6 ± 183.6

Average weekly mean power and peak power values over the eight weeks of exercise training presented as mean ± SD (n = 18). Mean power and peak power were significantly reduced over the training program (p < 0.05).

FIGURE 1

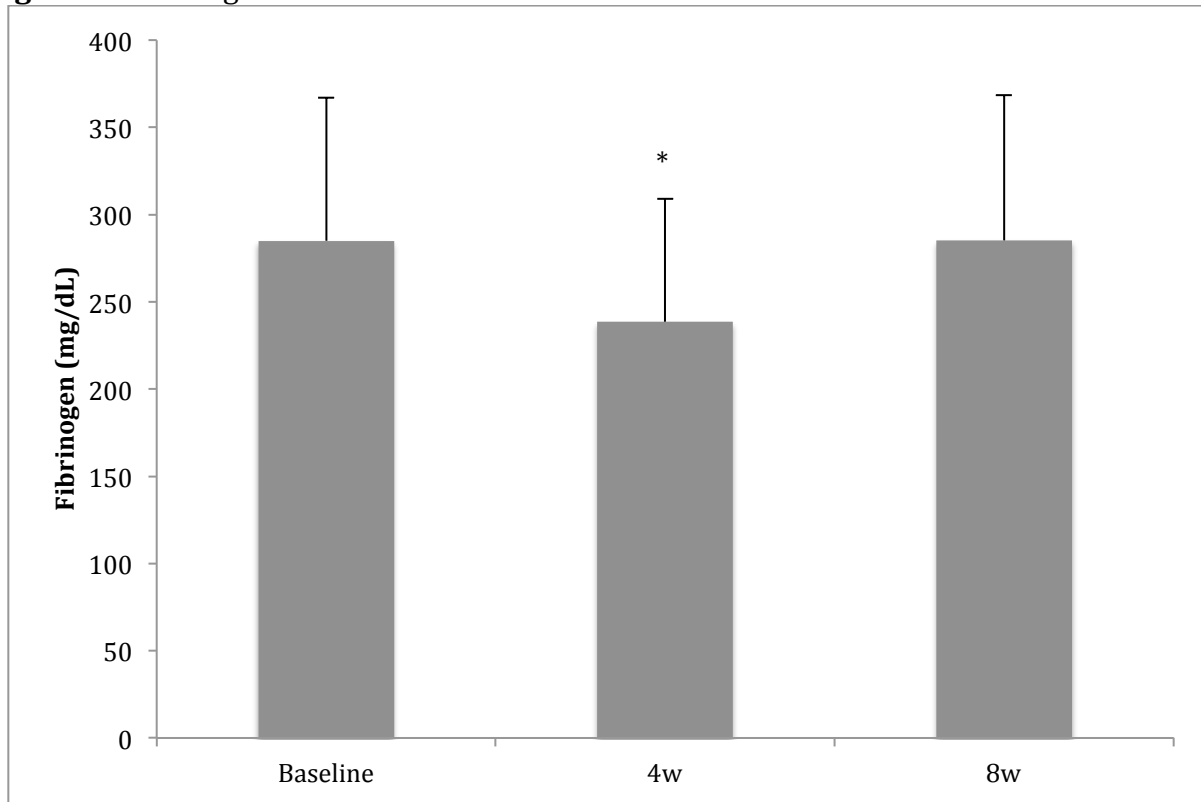
Figure 1. Training Stimulus of Fatigue Index



Average fatigue index for each week over the eight weeks of exercise training presented as mean \pm SD. Fatigue index was significantly reduced over the training program ($p < 0.05$).

FIGURE 2

Figure 2. Fibrinogen Concentrations



Fibrinogen levels at baseline, 4w, and 8w of HIIT presented as mean \pm SD. Fibrinogen concentrations were significantly decreased at 4w compared to baseline and 8w ($p < 0.05$).

CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

The purpose of the present study was to examine changes to the hemostatic system before, during, and after an eight week HIIT intervention. The major finding was that fibrinogen levels were significantly lower after 4w of HIIT compared to baseline, and significantly increasing over the remaining weeks of training, essentially returning to baseline levels. The significant decrease in fibrinogen from baseline to 4w supports our hypothesis that HIIT would cause a reduction in coagulation potential. Previous research indicates that fibrinogen levels are decreased following intense physical training [9, 73, 74]. However, the rise in fibrinogen after the eighth week of training (back to baseline levels) is not supported by our hypothesis. While this significant increase in fibrinogen levels was unexpected, it has previously been seen in the literature [75-77]. The increase in fibrinogen levels may be explained by the substantial increase in volume of exercise

training that occurred in the latter four weeks of training compared to the first four weeks of training.

Several studies have examined plasma fibrinogen concentrations following intense physical training programs and fibrinogen levels were shown to decrease [9, 74], increase [76, 77], or not change [78, 79] with different modes of training. Most of the previously mentioned training regimens that showed a decrease in fibrinogen levels entailed subjects who may have had increased levels of fibrinogen before the exercise training program because of links to obesity and/or previous cardiac events. Likewise, because of the increase in training volume, compared to normal everyday activity, those subjects experienced decreased fibrinogen levels. On the other hand, Ponjee et al. conducted a study in 1993 that showed similar results to our study [77]. While the training regimen was unique in the aspect that the subjects were training for a half-marathon race, they showed an initial decrease in fibrinogen levels followed by a significant increase in fibrinogen levels that mirrored the increase in training volume and intensity. Sedentary men and women trained at increasing intensities and volumes for 9-months in preparation for a race via long-distance running, running at high speeds, and interval training. An initial decrease in fibrinogen levels is seen at 13 weeks of training followed by a gradual increase in fibrinogen levels all the way through the end of the study. Similarly, another study showed an increase in fibrinogen in male army recruits following 10 weeks of basic training [76]. Although not specified in depth in the paper, one may expect the exercise protocol of basic training camp to be of extreme vigorous intensity and of high volume. Combining these results and the results of the present study, it is suggested that fibrinogen

levels may decrease following a moderate intensity and/or volume of exercise training but may increase following a high intensity and/or volume of exercise training.

The observed changes in fibrinogen levels in the present study may be related to the fact that fibrinogen is an acute phase reactant protein. This indicates that plasma concentrations of fibrinogen will increase or decrease parallel to inflammation. Furthermore, previous research has demonstrated that intense physical training exacerbates inflammation [114]. Plasma concentrations of fibrinogen generally range from 200 to 400 mg/dL [115], and fibrinogen levels in the present study remained within range throughout. In pathological conditions, such as injury or diseases associated with vascular disruption, fibrinogen levels have been shown to increase several-fold [116]. While its role in the coagulation cascade is well established, a growing body of evidence implicates fibrinogen concentration as a key regulator of inflammation [117]. An in depth review by Davalos & Akassoglou discuss how our understanding of fibrinogen is evolving from a building block of blood clots to an essential signaling molecule with a broad spectrum of functions, including extensive inflammation, eventually leading to life or death [117]. Fibrinogen signaling is becoming more and more evident through its binding sites that are non-overlapping with those in the coagulation cascade [117]. With the perspective that fibrinogen levels are related to inflammation, our data suggest that the first four weeks of training in this study did not cause chronic inflammation and therefore significantly reduced fibrinogen levels. This is comparable to what is seen in the literature with regards to traditional, aerobic exercise training [9, 73]. However, when the volume of exercise is ramped up (latter four weeks), it is theorized that chronic inflammation was stimulated,

leading to a significant rise in fibrinogen levels through its several binding sites as it acts as a mediator of inflammatory disease.

It is important to further understand the relationship between inflammation and exercise training because of the positive relationship between the several markers of inflammation and the risk for an adverse cardiovascular event in both healthy and diseased populations [118, 119]. One of the reasons that exercise training is shown to be beneficial to overall health is because it is known to attenuate inflammation [120] and the literature is convincing with regards to the idea that inflammation and exercise are inversely related [69, 79, 121-124]. Previously published studies observe several important markers of inflammation including fibrinogen, C-reactive protein, interleukin-6, and adhesion molecules.

Koenig and colleagues conducted an eight-year study and concluded that C-reactive protein is a sensitive systemic marker of inflammation and related to physical activity [121]. C-reactive protein levels were positively correlated with the incidence of an adverse cardiovascular event. Due to the length of this study, subjects were of various levels of fitness and some fitness levels changed drastically over the time period of the study. Likewise, Rohde et al. conducted a few studies that showed that C-reactive protein and interleukin-6 are good prognostic markers of inflammation with regard to exercise training and in turn related to the risk of an adverse cardiovascular event [122, 123]. While observing an elderly cohort, another study showed an inverse relationship between several inflammatory markers (e.g., fibrinogen, FVIII, C-reactive protein) and physical activity [69]. On the other hand, a study done by Marcell et al. did not show improved levels of C-reactive protein following exercise training [125]. This study observed healthy but overweight

individuals over 16 weeks of moderate and intense exercise training. While the findings of the previously mentioned studies are compelling, they differ from the present study due to the fact that most of them observed changes following an extended period of time (i.e., years). However, because HIIT is a newer mode of exercise training and still only short term, the findings are not as convincing.

While we theorize that chronic inflammation is induced following HIIT, there is a lack of previous research to confirm this idea and the minimal literature on inflammation and HIIT actually states the opposite. A study done by Munk et al. observed post percutaneous coronary intervention patients and showed that some, but not all, inflammatory pathways were attenuated following HIIT [21]. Furthermore, Peschel et al. observed a reduced expression of adhesion molecules in response to a high intensity training program and correlated the reduction in inflammatory markers to the intensity of exercise [126]. The similarity between the aforementioned studies lies within the subject population. The subjects studied were of the cardiac or diseased population and they may have obtained increased inflammatory components prior to exercise training. These studies denote the need for future research with regards to the relationship between inflammation and HIIT in the healthy population. Because fibrinogen is more commonly being related to inflammation and disease [127], we speculate a positive relationship between HIIT and inflammation. We believe this because the subjects of the present study were apparently healthy with no contraindications for exercise training and not of the diseased population, like the studies mentioned above. Due to our findings in the present study, we believe that inflammation may parallel an increase in exercise training (e.g., higher intensity and volume).

While the rise in fibrinogen levels observed in the final four weeks of this study is still important with regards to thrombosis, it is postulated that this adaptation did not directly affect the coagulation cascade, a hypothesis that is supported by the results of the clotting time analyses. Unlike fibrinogen, no significant differences were found with regard to APTT between baseline, 4w, and 8w. This is the first study to our knowledge that showed no changes in APTT following an intense exercise training program. Previously published articles showed APTT either increases [10, 71] or decreases [14, 63] with exercise training. With the significant changes in fibrinogen levels, one may expect to see APTT levels change as well because fibrinogen is a part of the coagulation cascade's intrinsic pathway, which is evaluated during an APTT test. However, a change in fibrinogen levels does not always affect the coagulation cascade. As mentioned above, the rise in fibrinogen may have been through binding sites that are not involved in the coagulation cascade, and related to inflammation [117]. This suggests that while fibrinogen levels increased in our subjects, it may not have actually affected coagulation activity, as APTT's did not change over the entirety of the study.

Similarly to APTT, no significant difference was found in PT between baseline, 4w, and 8w. However, contrary to the measure of APTT, a PT test does not assess the portion of the coagulation cascade in which fibrinogen plays a role. The fact that PT did not change throughout the training program is shown in the literature and confirmed with this study. Several investigations in the past have analyzed PT's and shown that exercise training does not affect it [12, 63, 71, 128]. These studies encompassed different modes and different volumes of exercise as well as different populations of subjects. This finding, which is

comparable to what is seen in the literature, suggests that the extrinsic pathway is not a modulator in the coagulation changes associated with exercise training.

A limitation of this study was our subject population of college-aged men. Coagulation parameters differ significantly based on the population of the cohort and our subject population may not be generalizable to the other populations. It is thought that clinical and cardiac populations that may already have an elevated inflammation and/or coagulation potential might experience something different during HIIT. Another limitation of this study lies within the fact that we did not objectively measure a training adaptation. While it is believed that a training adaptation occurred, no improvements were shown via a Wingate test. Therefore, future research may want to measure training adaptations via a single Wingate test or VO_2 max test before and after the training program. Finally, the lack of a non-exercise or endurance training control group may have limited our ability to observe significant adaptations to HIIT. These groups were not incorporated because of the lack of available time commitment of the research team.

Future research should focus on the goal to elucidate the volume of HIIT required to elicit beneficial improvements in blood clotting parameters. Specifically, it is important to find the volume and intensity of an exercise training program that yields the most beneficial values with regards to fibrinogen. Careful investigations need to be conducted with regards to the putative time course of adaptations and exercise intensity/volume. Several studies used six weeks of HIIT and showed positive changes [92, 98], but our eight week study did not concur. It is also suggested that future investigations extend HIIT into 10, 12, and more weeks to analyze the occurring adaptations. Furthermore, future research should explore inflammation in response to HIIT including other markers of

inflammation such as interleukin-6, C-reactive protein, interleukin-1, and/or adhesion molecules.

Conclusion

In conclusion, the present study indicates that a four-week HIIT program may reduce fibrinogen levels, but an additional four weeks of HIIT brought fibrinogen levels back to baseline values. It is unclear if continued HIIT would provoke further fibrinogen increases. This study elicited no change in APTT and PT values following four and eight weeks of HIIT. It is believed that blood clotting potential wasn't affected and that fibrinogen changes were due to an inflammatory response. While fibrinogen is an important marker of blood clotting in the coagulation cascade, it is becoming a more important mediator of inflammatory disease [117]. Increased fibrinogen levels are commonly found in cardiac patients, and an important risk factor for stroke [129, 130]. This suggests that, because of HIIT's ability to induce inflammation, HIIT may be dangerous for those with increased levels of fibrinogen and should be avoided by subjects at risk. Results of this study may be important for medical and exercise professionals who work with individuals with elevated inflammation and high thrombotic risk.

APPENDICES

APPENDIX A – Informed Consent

Consent Form: Hemostatic and Cardiovascular Adaptations Following a High-Intensity Interval Training Program in Healthy Men

The purpose of the present study is to examine the effects of a high-intensity interval training intervention on the following outcome variables that are independently associated with cardiovascular risk: (a) blood coagulation potential; (b) arterial distensibility; and (c) carotid intima media thickness.

Regular exercise is known to improve various measures of cardiovascular health. Traditional exercise recommendations include doing 20-60 minute bouts of continuous exercise, 3-5 days per week, at a moderate intensity. New research suggests that engaging in brief, repeated bouts of high-intensity activity may be as effective or even more effective than traditional exercise with regard to improving health and athletic performance. We are investigating whether this type high-intensity interval training will cause beneficial changes related to blood flow, blood clotting, and the way your arteries function, all of which are independently related to risk of a number of chronic illnesses and adverse outcomes, such as heart attack and stroke. The results of this study may provide important information regarding the usefulness of a high-intensity training program for improving cardiovascular health.

Healthy, non-smoking men who are between the ages of 18 and 45 years old and at least 50 kg (110 lbs) may be eligible to participate in this study. Participants must have no physical limitations that impact the ability to exercise, and should not be taking any medications that may influence the results of the study. You may be eligible if your exercise routine is irregular, including fewer than two days a week for the previous three months. This study includes exercise training for eight weeks, three days per week. All exercise training will be done in our laboratory under the supervision of study personnel. Training sessions will begin with a brief warm-up period, after which you would engage in a series of high-intensity training bouts. Each bout will consist of 30 seconds of high-intensity cycling followed by 4.5 minutes of easy walking or cycling. During the first two weeks, training sessions will include three bouts of cycling. With each successive two-week period, an additional bout will be added to the session, so that four bouts will be done per session in weeks 3-4, five bouts in weeks 5-6, etc. Each training session is expected to last less than 30 minutes.

You will be asked to report to the laboratory for data collection on three separate occasions. These visits will occur prior to the first week of training, after the 4th week, and then after the eighth week of training. You should wear shorts and a t-shirt for these three visits. Please avoid caffeine, exercise, alcohol, and nicotine for at least 24 hours prior to each data collection visit. You will also be asked to have fasted for the previous 12 hours. During data collection, we will measure height and weight, and then have you sit quietly for fifteen minutes. Resting heart rate, resting blood pressure, an electrocardiogram, ultrasound images of your femoral artery (near your groin) and carotid artery (side of your neck), and a 10-ml (<1 Tbsp) blood sample will be collected after the seated rest. The electrocardiogram and ultrasound image will be used to determine cardiovascular risk factors such as carotid intima media thickness and pulse wave velocity. The 10-ml blood sample will be obtained from a vein in your arm and will be used to measure blood clotting activity.

As with any physical exercise training, there is potential for prolonged muscle joint or soreness, and a slight risk of more serious muscular (e.g. muscle strain/sprain) or cardiovascular (e.g. heart attack, stroke) injury involved with this study. A recent study showed that this type of training was considered to be low risk for cardiac patients. Those high risk patients are expected to experience one overall adverse event in 23,182 hours of training. We expect the risk to be even lower for the apparently healthy participants in this study. Investigators will take measures to prevent such injuries, including

ensuring adequate warm-up and monitoring your responses during exercise. Risks of blood drawing may include discomfort, bruising, and, in rare cases, infection, lightheadedness, and fainting. We will use sterile procedures and trained personnel to ensure that there is minimal discomfort with obtaining the blood samples. Emergency medical treatment is available in the event of injury. You will assume responsibility for the costs of medical care that is provided. In the unlikely event of injury or illness of any kind as a result of participation in this research project, Ball State University, its agents and employees will assume whatever responsibility is required by law.

After you complete the study, you will be given any information collected as part of your participation in this project. We also anticipate you will experience a number of health benefits from participating in the training program. You may also benefit from the creation of new scientific knowledge regarding future exercise recommendations for healthy men.

Your participation in this research study is completely voluntary and you are free to withdraw from the study at any time for any reason without penalty or prejudice from the investigators. Please feel free to ask any questions to the investigator before signing this Informed Consent form and beginning the study, and at any time during the study. All study materials and information will be maintained as confidential. Your information will be stored for five years after completion of the study. Data will be stored in a locked filing cabinet in the researcher's office. Electronic files, which will be password protected, will be stored on the principal investigator's computer and preferred backup medium. If you are not eligible to participate in this study your information will not be retained, and will be destroyed by shredding paper files and permanently deleting electronic information.

For one's rights as a research subject, contact the Director, Office of Research Integrity, Ball State University, Muncie, IN, (765) 285-5070 or the Institutional Review Board Administrator at Ball State University at irb@bsu.edu.

I, _____, agree to participate in this research project entitled, "Hemostatic and Cardiovascular Adaptations Following a High-intensity Interval Training Program in Healthy Men". I have had the study explained to me and my questions have been answered to my satisfaction. I have read the description of this project and give my consent to participate. I understand that I will receive a copy of this informed consent form to keep for future reference.

Participant's Signature

Date

Investigator's Signature

Date

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APPENDIX B – Health History Questionnaire

Please mark all *true* statements:

History

You have had:

- ☐ A heart attack
- ☐ Heart surgery
- ☐ Cardiac catheterization
- ☐ Coronary angioplasty (PTCA)
- ☐ Pacemaker/implantable cardiac
- ☐ Defibrillator/rhythm disturbance
- ☐ Heart valve disease
- ☐ Heart failure
- ☐ Heart transplantation
- ☐ Congenital heart disease

Symptoms

- ☐ You experience chest discomfort with exertion.
- ☐ You experience unreasonable breathlessness.
- ☐ You experience dizziness, fainting, or blackouts.
- ☐ You take heart medications.

Other Health Issues

- ☐ You have diabetes.
- ☐ You have asthma or other lung disease.
- ☐ You have burning or cramping sensation in your lower legs when walking short distances.
- ☐ You have musculoskeletal problems that limit your physical activity.
- ☐ You have concerns about the safety of exercise.
- ☐ You take prescription medication(s).

Cardiovascular Risk Factors

- ☐ You are a man older than 45 years.
 - ☐ You smoke, or quit smoking within the previous 6 months.
 - ☐ Your blood pressure is > 140/90 mm Hg.
 - ☐ You do not know your blood pressure.
 - ☐ You take blood pressure medication.
 - ☐ Your blood cholesterol level is >200 mg/dL.
 - ☐ Your good cholesterol (HDL) is <40 mg/dl *OR* your bad cholesterol (LDL) is >130 mg/dl
 - ☐ You do not know your cholesterol level.
 - ☐ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
 - ☐ You are physically inactive (i.e., you get <30 minutes of physical activity on a least 3 days per week).
 - ☐ You are >20 pounds overweight.
 - ☐ None of the above is true.
-

Exercise History: Briefly describe your typical exercise routine. Specify the type (*e.g.* aerobic, resistance) and number of hours (per day, days per week) you usually exercise:

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